

ANATOMICAL STUDIES OF BARK REGENERATION FOLLOWING SCORING

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SCORING of the trunks or branches of fruit and ornamental trees to induce earlier and more prolific flowering and fruiting has been practised for thousands of years. This method of checking phloem transport is more temporary in its effect than the ancient procedure of ringing or the more recent bark inversion (Sax, 1954), but it is easily done and is less hazardous than either of these practices. If scoring does not inhibit phloem transport long enough to insure maximum response, it can be repeated. In order to determine the duration of the effect of scoring and the nature of bark regeneration, studies were made with both slow- and rapid-growing trees.

Two types of trees were used, the fast-growing silver maple (*Acer saccharinum*) and a hybrid poplar (ex *Populus deltoides*), and the slow-growing apple, 'Prairie Spy,' and the 'Seckel' pear. The maple and poplar trees were three years old while the pear and apple trees were five to six years old. The trunks and branches were scored by cutting with a knife through the bark to, and often into, the underlying xylem. In the earlier experiments, the cuts were made directly around the trunk or branch, but in the later the cut was made in a diagonal spiral to prevent the breakage of branches. Samples of the cut bark and underlying wood were taken every few days after scoring in order to study the regeneration of the severed bark. After fixing, longitudinal radial sections were cut, stained and mounted.

HISTOLOGICAL TECHNIQUE

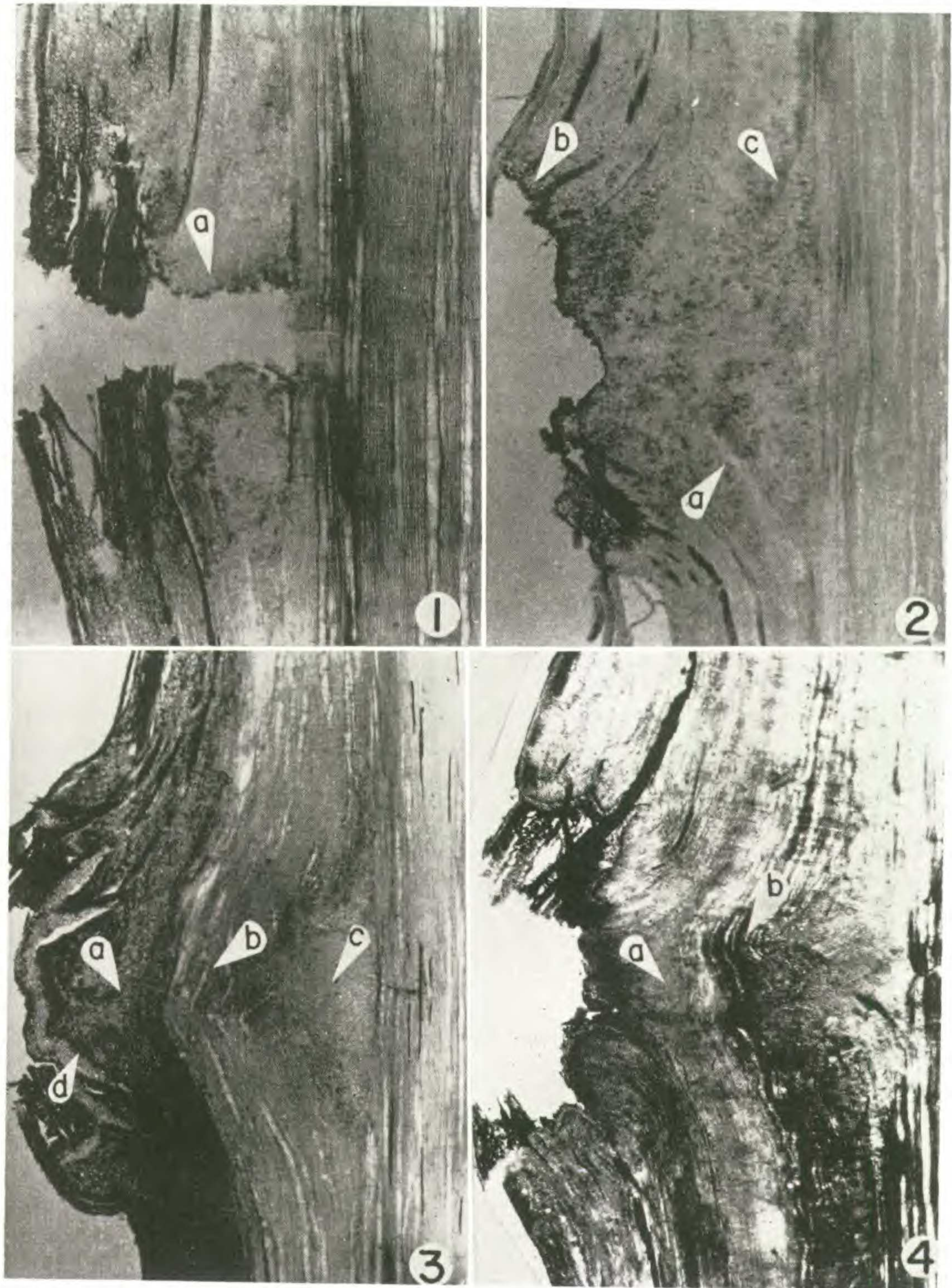
Samples removed from the trees were treated in two ways. In most of the cases, the samples were cut into 15–20 μ sections on a sliding microtome. The majority of the sections were radial longitudinal sections, but cross sections were cut when necessary. For more detailed studies of the material removed from the trees, it was fixed in formalin acetic alcohol (Johanson, 1940). A modified Zirkle's n-butyl alcohol method for dehydration was employed and the paraffin-tissuemat method of Pratt and Wetmore (1951) was used for embedding the material. Just before microtoming, the embedded material was exposed by cutting off the unnecessary paraffin on the required surface and soaked in 50% alcohol for at least three hours before cutting. To avoid curling and to insure easy handling of the sections a thin sheet of polyethylene was used to cover the top of the material before each section was cut. The flattened sections on the polyethylene sheet were mounted on cleared slides smeared with egg-albumen fixative and flooded with 2% formalin.

Different stain combinations for studying different elements during the

development of the new bark were used as follows: (a) 1% safranin counter-stained with 0.5% fast green in 100% ethyl alcohol and clove oil (Johanson, 1949); (b) 1% safranin with 2% analin blue in methyl cellosolve; (c) bismark brown, iodine green, resorcin blue (lacmoid) (Esau, 1948) all diluted 1/5,000 (Esau, 1948).

OBSERVATIONS

Poplar. The three-year-old poplar stem has a thick cortical region and the phellem of the normal bark consists of five to ten years of cork cells. In the phloem, the sieve elements were in patches and were arranged in alternate tangential rows with phloem fibers, each row being of one to eight phloem fibers thick. The cambial zone (vascular) with its immediate derivatives formed a distinct zone. When studied after scoring, it was shown that the ringing reached into the mature xylem two to ten cells (or approximately so) deep. By the third day, the exposed cut surfaces of the wound had dried out, especially the marginal cells in the region of cork, cork cambium, cortex and outer mature phloem cells. Callus formation took place at this stage from two living tissue systems: (a) the living cells of the longitudinal conducting system which includes phloem parenchyma, vascular cambial zone and xylem parenchyma; (b) the horizontal living cells which comprise the vascular rays of the phloem, the vascular cambium, and both immature and mature xylem (FIGS. 5, 6). On the sixth day, there was very active callus formation and the two edges of the callus formed on the upper and lower margins of the cut fused (FIG. 2) and bulged out. The outermost cells of the callus were exposed and dried out. Some of the immature xylem elements near the wounds became mature while callus was forming. They became lignified and were characteristically distorted as they were pushed out by the increasing volume of callus below them, especially the active growth of the vascular ray cells (FIG. 2). Just outside of these lately-formed, distorted xylem elements, a series of cells within the callus developed into a distinct cambial zone which formed a bridge over the cut (FIG. 2). This formation of a new cambial zone began at the regions where the callus tissue met the normal undisturbed cambial zone. At the deepest part of the cut, mature xylem cells characteristically show the lumen filled with cells which closely resemble tyloses. The entrance of these cells into the vessels has not been observed, but all indications seem to point to their being tylose in nature, originating from neighboring ray initials or callus cells formed from such ray initials. Practically every mature vessel in the neighborhood of the bottom of the cut is so filled. While some of the xylem elements became distorted as they matured, the mature phloem which was not taking part in the formation of callus was pushed outward. Some phloem tissue entered into the formation of callus. On the ninth day, it was found that the newly formed vascular cambium had produced new xylem elements of a short and distorted nature towards the inside. Also, phloem elements were formed outwards as usual, and these were also distorted and abnormal in their mor-



FIGS. 1-4. Development of callus and new vascular cambium, cork cambium, phloem and xylem at the region of the cut after scoring.

FIG. 1. Radial longitudinal section of poplar stem at the region of the wound, three days after scoring: a, callus formation is initiated on either side of the wound in the region of immature phloem, vascular cambium and immature xylem ($\times 24$). FIG. 2. Radial longitudinal section of poplar stem at the region of the wound, six days after scoring: a, vascular cambium; b, cork cambium; c, some immature vascular elements become mature after being pushed out by the growth of callus tissue ($\times 21$). Note that the callus tissue from both sides of the wound fuses to form a continuous mass. FIG. 3. Radial longitudinal section of poplar stem sixteen days after scoring: a, region of new phloem; b,

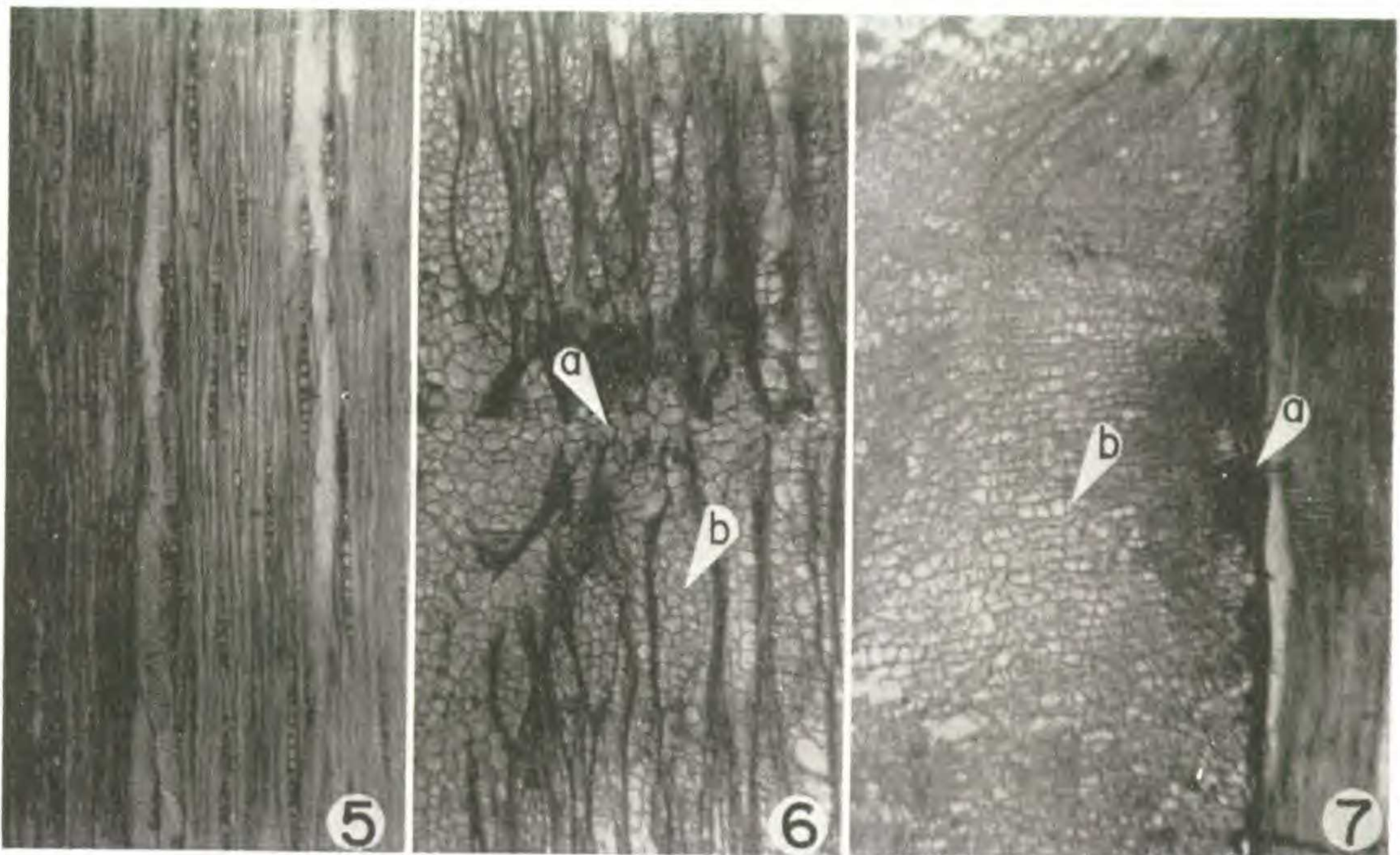
phology. Some sieve-tube elements even showed the presence of callose formation. The phloem fibers, instead of showing their normal elongated form, took the form of rounded sclereids. By this time a new cork cambial layer was well established in the callus region outside of the vascular cambium where it joined the normal cork cambium zone on either side, thereby becoming continuous over the wound. From the twelfth day to the sixteenth day, the xylem elements produced gradually assumed their normal characteristics in size and form, while the newly formed phloem elements were still abnormal in their morphology (FIG. 3). However, on the twentieth day the new vascular system, phloem as well as xylem, and periderm formation became perfectly normal.

Maple. In a three-year-old maple stem, the bark is thinner than in the poplar. The observations made on bark regeneration were more or less comparable to those of the poplar. On the third day after scoring, the usual drying of the cells in the exposed area took place in the cork, cork cambium, phloem and even the immature xylem elements. Callus was actively forming by the sixth day; it was comparable to the callus formation of poplar as described. On the ninth day, the two callus masses, one above and one below the wound, fused, and a distinct vascular cambial layer was formed in the callus which united the two edges of the undisturbed vascular cambium. On the twelfth day, the vascular cambial zone was well established and began to produce its derivatives, but no mature vascular elements were found as yet. By the sixteenth day, the mature products of the newly formed vascular cambium became distinct, both xylem and (less distinctly) phloem. Although the earlier-formed xylem elements were short, distorted and irregularly arranged, the later ones became more normal. Newly formed phloem elements were more distorted, irregular in arrangement, and fewer than the newly formed xylem elements. By the twentieth day, the vascular cambium was producing perfectly normal phloem and xylem elements.

Apple. The apple variety we used ('Prairie Spy') had a green bark which was thicker than that of the poplar mentioned above. On the third day after scoring, there was no obvious change, but there was a slight callus formation in the region of immature phloem and xylem along the flanks of the cut. On the sixth day, there was more drying of the exposed edges of the wound and there was a more obvious production of callus which originated in a manner similar to that in the previously mentioned plants. At this time, a layer of living cells which was below the dried cells at the regions of cork cambium, cortex and outer portion of the phloem became active and acted as a new cork cambium. On the ninth day, the callus formation became faster than before and the two edges of

region of newly formed xylem elements; c, callus cushion between the new and old xylem; d, new cork cambium ($\times 21$). FIG. 4. Radial longitudinal section of stem of apple twenty days after scoring, showing resumption of normal vascular and cork cambial activities: a, region of newly formed phloem; b, region of newly formed xylem ($\times 18$). Compare the amount of callus produced in apple (FIG. 4) with poplar (FIG. 3).

callus tissue fused together. There was a definite cambial zone formed which bridged the cut, but no obvious vascular elements were formed as yet. By then the cork cambium had produced four or more layers of derivatives. On the twelfth day, the callus formation did not show any progress and thus the amount of callus formed did not even reach to the level of the epidermis of the axis (FIG. 4). The hidden small amount of callus which developed and differentiated to produce both cork cambium and vascular cambium in no more time than in the abundant callus of poplar and maple, was very deceptive when one examined the wound superficially. But, remarkably, the vascular cambium had already been producing both phloem and xylem composed of short, distorted and irregularly arranged elements, and by the sixteenth day, the production of normal phloem and xylem was resumed. On the twentieth day, the newly formed vascular cambium and cork cambium were perfectly normal and were producing normal derivatives (FIG. 4).



FIGS. 5-7. Callus development from cells of the rays after scoring. FIG. 5. Tangential longitudinal section of poplar showing the characteristic uniseriate rays of a normal plant ($\times 36$). FIG. 6. Tangential longitudinal section of stem of poplar at the region of the wound, showing the multiseriate ray cells which take a major part in the formation of callus: a, the region of the cut made by the knife in scoring; b, multiseriate ray with large component cells ($\times 36$). FIG. 7. Radial longitudinal section of stem of maple showing callus tissue being derived from the ray cells: a, vascular ray cells continuous with their derivative callus cells; b, callus cells which have divided by successive periclinal divisions to form horizontal rows of callus cells ($\times 36$).

Pear. In pear, the nature of bark regeneration was almost the same as the apple. The callus formation began on the sixth day, and it was relatively small in comparison with the poplar and maple. On the twelfth day, the vascular cambium was produced and the new cork cambium was well

established. By this time, the vascular cambium was producing short and distorted vascular elements. By the sixteenth day, the production of the normal vascular tissue was resumed and by the twentieth day, both cork cambium and vascular cambium resumed their normal functions perfectly and produced derivatives which were normal in structure, size and arrangement.

DISCUSSION

The present investigation indicates that the amount and the rate of callus production in a wounded area following scoring varies in the different plants studied. The source of callus also varies to some extent. In scoring experiments on trees of poplar, silver maple, pear and apple, callus formation is mainly contributed by living cells of vascular rays (Figs. 5, 6, 7) in the proximity of the cut. There is also some evidence that living longitudinally oriented parenchyma cells of phloem, of xylem and of vascular cambium take part in the formation of callus (Fig. 1). If we look at the literature on the source of callus in the regeneration of new bark, well discussed by Bloch (1941), one finds a diversity of opinions on the origin of callus. Sass (1932) in his study of the formation of callus knots on apple grafts showed that it is produced exclusively by tissues located outside of the xylem cylinder. According to him, any living tissue of the bark, excluding the periderm, may proliferate, and the cambium may produce very little callus. On the other hand, Sharples and Gunnery (1933) showed that in their study of the development of callus in the healing of a surface wound produced by an excision of a strip of bark from the stem of *Hibiscus rosa-sinensis* and *Hevea brasiliensis* (1933), the development of the callus cushion is predominantly from the vascular ray system and the cambium takes no part in its early stage of development. It is clear that Sass in his investigation showed the origin of callus from all the living tissues of the bark except periderm, while Sharples and Gunnery showed that the vascular ray cells are the main source for the origin of callus. The latter are supported by observations made in the present investigation (Figs. 5, 6, 7). Both of them agree that the cambium took very little part in callus formation which is also supported by the present investigation.

In this study it was found that new vascular cambium formation took place on the sixth day in poplar and on the ninth day in maple, pear and apple trees. The new vascular cambium formation is usually independent of the amount and the rate of callus production. It was also found that the activity of the new cork cambium started as soon as the wound callus pad is well developed. The formation of cork cambium was always found ahead of the vascular cambium formation in the plants studied.

In the present investigations, the new xylem elements, followed quickly by the new phloem elements, started appearing soon after the formation of the vascular cambium (that is, about on the ninth day) but they were not abundant and all were abnormal and distorted. In some cases, callose material was seen in and near plates of the sieve-tube elements even on

the ninth day. It was only on the twentieth day, or approximately so, that the plants studied showed normal and regular development of xylem and phloem elements. However, as before the scoring, the number of xylem elements produced was naturally more than that of the phloem. It is interesting that the amount of callus formed in poplar and maple was more and appeared earlier than in pear and apple; yet all four produced new phloem and xylem at about the same time. The result of the present investigation on bark regeneration agrees with that of Murneek (1939), in which he states that the branches of apple trees ringed with a wire girdle healed in three to five weeks.

The anatomical studies of spiral ringing in crab apple and branches of other apples showed that the conducting tissue formed by the cambium subsequent to the scoring was changed in orientation so that the long axes of the elements were parallel to the spiral.

CONCLUSION

Following scoring, the new cambium is formed in about a week, but the complete restoration and functioning of the phloem required about three weeks. If done at an appropriate time of year, a single scoring may check phloem transport long enough to induce flowering and fruiting, but, if not, the process can be repeated every two or three weeks to insure earlier flowering and fruiting of ornamental and fruit trees.

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